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## MINI SYMPOSIUM: PET—THE FUTURE

Tuesday 17 October 2006, 14:00–15:30

# From anatomical to biological target volumes: the role of PET in radiation treatment planning

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### Abstract

Progress in radiation oncology requires a re-evaluation of the methods of target volume delineation beyond anatomical localization. New molecular imaging techniques for tumour visualisation such as positron emission tomography (PET) provide insight into tumour characteristics and can be complementary to the anatomical data of computed tomography or magnetic resonance imaging. In this review, three issues are discussed: First, can PET identify a tumour more accurately? Second, can biological tumour characteristics be visualised? Third, can intratumoural heterogeneity of these characteristics be identified?

**Keywords:** Radiation treatment planning; PET; target volume.

### Introduction

In the past decade, there has been substantial technological progress in radiation oncology. Dose delivery with high geometric precision is possible due to the introduction of stereotactic radiotherapy, radiosurgery, intensity modulated radiotherapy (IMRT) and three-dimensional planning of brachytherapy. These developments require a re-evaluation of the standard methods for target volume delineation. The current standard of target volume definition is based on information gathered by physical examination, computed tomography (CT) and magnetic resonance imaging (MRI). In recent years new methods for tumour visualisation have been introduced in oncology. Imaging techniques such as positron emission tomography (PET), single-photon emission computed tomography (SPECT) and magnetic resonance spectroscopy (MRS) are able to visualise biological characteristics of tumours, providing information on metabolism, physiology and molecular biology of tumour tissue. These so-called 'functional' or 'molecular' imaging modalities complement the anatomical data supplied by CT and MRI and include several potential advances. First, the primary tumour can be identified more accurately. If carefully validated this could resize and reshape the gross

tumour volume (GTV). This consequently could increase cure rates by reducing the chance of geographically missing part of the tumour during the treatment. When imaging modalities become more accurate, the inter- and intraobserver variation in tumour delineation decreases, which implies an enormous increase in the standard of care. More accurate tumour identification could also lead to an increased normal tissue sparing. Second, tumour characteristics relevant for radiation sensitivity can be visualised. Functional imaging may identify the degree of radiosensitivity of tumours, leading to an individualization of the radiation treatment. For example, the addition of a hypoxic cell sensitiser like nimorazole could be advantageous for radiation treatment when the tumour demonstrates a certain level of hypoxia<sup>[1]</sup>. Third, intratumoural biological heterogeneity can be identified. Ling *et al.*<sup>[2]</sup> introduced the concept of 'biological target volume'. This biological target volume represents a subvolume of the tumour with specific characteristics on functional or molecular imaging techniques. Subvolumes that are relatively resistant to radiation receive an extra dose delivered with high precision on a small volume, which is called 'dose painting' or 'dose sculpting'. This could increase cure rates without increasing the chances of late radiation-induced toxicity.

In this review we focus on PET as this molecular imaging technique is becoming widely available at an astonishing rate. Significant clinical work has already been done to investigate its possible role in radiation treatment planning. The following three issues are discussed:

- (1) Can PET identify the primary tumour more accurately?
- (2) Can biological tumour characteristics be visualised?
- (3) Can intratumoural biological heterogeneity be identified?

### Can PET identify the primary tumour more accurately?

#### Co-registration

CT is the reference imaging modality for radiation treatment planning as it provides electron density information of the various tissues which is needed for the dose calculation algorithms. However, CT images lack contrast between soft tissue structures and tumour extension. For example, the assessment of oral cavity and oropharyngeal tumours is severely hampered by scatter artefacts of dental fillings. Compared to CT, MRI has shown to be more accurate in evaluating soft tissue and can be more sensitive for bone invasion of head and neck tumours, but it also has its limitations, such as geometric distortions at field of view edges, and artefacts at interfaces of bone and air.

The observation that malignant tumour cells are characterised by increased glycolysis resulted in the development of whole body imaging using PET and the fluorine-18 labelled glucose analogue fluorodeoxyglucose (FDG). The high sensitivity of FDG-PET is related to the upregulation of glucose transporters on the cell membrane as well as increased hexokinase activity in a wide variety of malignancies<sup>[3]</sup>. Following phosphorylation by hexokinase, FDG is trapped in cells and leads to an uptake into tissue in proportion to the overall glucose metabolism. FDG uptake, however, is not cancer-specific, as increased glucose metabolism is also seen in inflammatory processes, muscle activity and brain activity.

To incorporate PET data into CT-based tumour delineation there are three options of image fusion: visual fusion, where the physician compares two separate imaging modalities viewed next to each other, software fusion, where both modalities acquired on separate machines are overlaid in an integrated set of images (co-registration), and hardware fusion in which both data sets are acquired on one single machine, e.g. a hybrid PET/CT scanner.

The co-registration of CT (and/or MRI) and PET images has successfully been demonstrated by many

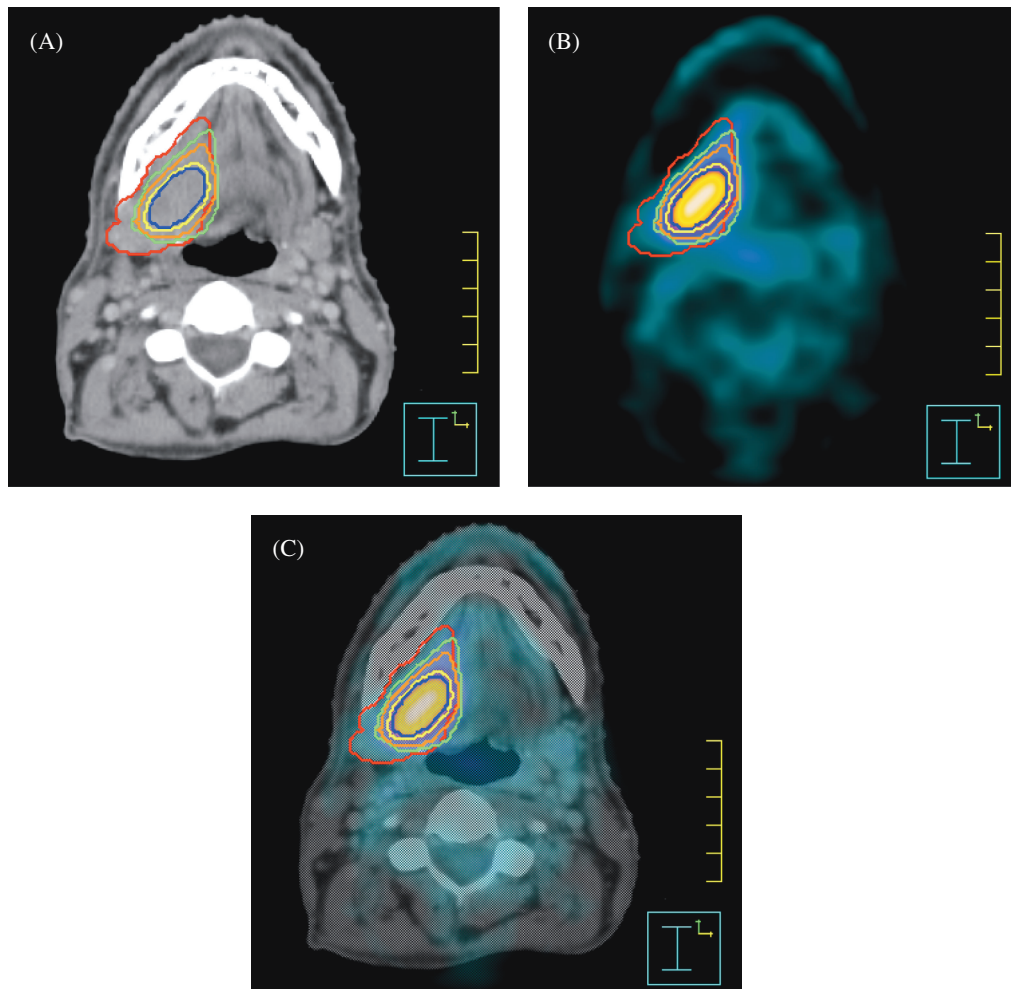
groups<sup>[4–6]</sup>. If performed properly, co-registration matches the performance of a hybrid PET/CT<sup>[6]</sup>.

#### Validation

In 2005 Ng *et al.*<sup>[7]</sup> reported on 124 patients with cancer of the oral cavity, all eligible for surgery. Non co-registered FDG-PET and CT or MRI were obtained to determine their performance in detecting the primary tumour with histopathology as the gold standard. This, the largest study to date, confirmed earlier data showing that 122 of 124 tumours were correctly detected by FDG-PET versus 108 on CT or MRI. FDG-PET missed a small superficial tumour and misinterpreted a floor of mouth tumour for a tongue tumour. However, no attempt was made to accurately delineate the tumour. The best evidence to date that FDG-PET can identify the primary tumour in head and neck cancer more accurately than conventional imaging is provided by Daisne *et al.*<sup>[8]</sup>. They compared the role of co-registered CT, MRI and FDG-PET in delineating the primary tumour in nine patients with laryngeal cancer who were scheduled for laryngectomy. Compared to the reference surgical specimen, all modalities overestimated the extension of the tumour. The average GTV on histological examination was 12.6 cm<sup>3</sup>, whereas averages for the various imaging modalities were 16.3 cm<sup>3</sup> (PET), 20.8 cm<sup>3</sup> (CT) and 23.8 cm<sup>3</sup> (MRI). PET was closest to depict the true tumour volume, but all three imaging modalities (including PET) failed to identify a small fraction (approximately 10%) of the macroscopic tumour, mainly superficial mucosal extension.

Application of radiopharmaceuticals other than FDG which depict different characteristics of tumour cells may also play a role in accurate tumour definition, e.g. the amino acid based radiopharmaceuticals *O*-[2-<sup>18</sup>F fluoroethyl]-L-tyrosine (FET) and [methyl-<sup>11</sup>C]methionine (MET). In brain tumour imaging FET and MET both have an advantage over FDG, as they do not accumulate in normal brain cells. FET-PET was superior in delineating human gliomas compared with MRI, which was demonstrated by the use of stereotactic biopsies in 28 glioma patients<sup>[9]</sup>. When comparing FET-PET to FDG-PET and CT in 18 patients with head and neck cancer, both FET-PET and FDG-PET were superior to CT in the detection of tumour<sup>[10]</sup>. FET-PET showed no uptake in physiologic tissue and inflammatory tissue, resulting in a higher specificity for tumour detection than FDG-PET. A drawback of FET-PET was a lower sensitivity, caused by a relatively low tumour uptake compared to FDG-PET.

MET-PET images in patients with brain tumours were investigated by correlating MET uptake with histological examination of stereotactic biopsies<sup>[11]</sup>. Solid parts of brain tumours as well as brain tissue with infiltrating tumour cells were detected with high sensitivity (87%) and specificity (89%). Kracht *et al.*<sup>[11]</sup> emphasized its



**Figure 1** Planning CT scan (A), corresponding FDG-PET scan (B) and fusion image (C) show differences in target volume definition. Volume GTV CT (red) = 47.5 cm<sup>3</sup>, GTV VIS (green) = 43.8 cm<sup>3</sup>, GTV 40% (yellow) = 20.1 cm<sup>3</sup>, GTV 2.5 (orange) = 32.6 cm<sup>3</sup>, GTV UCL (blue) = 15.7 cm<sup>3</sup>. Note that GTV UCL is significantly smaller than GTV CT and GTV VIS.

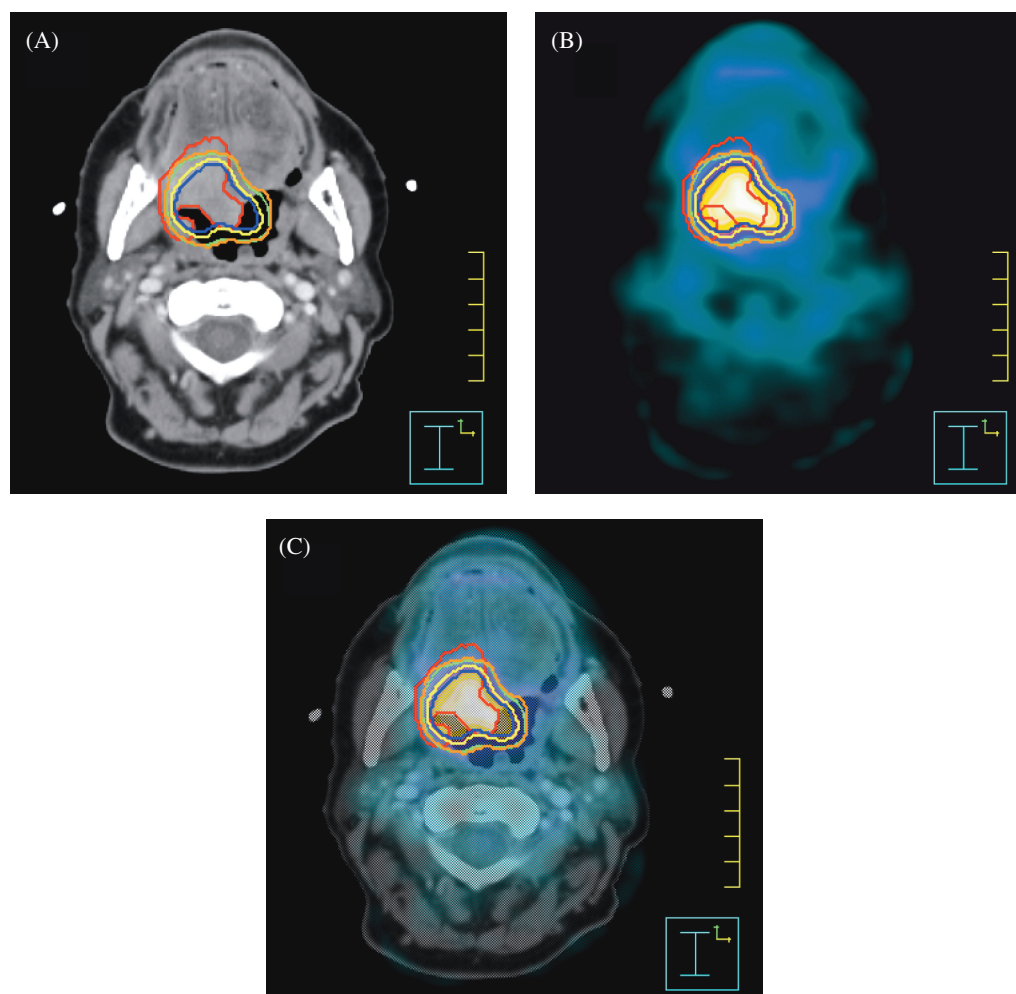
value as a delineation tool for treatment planning in neurosurgery and radiotherapy. Grosu *et al.*<sup>[12]</sup> investigated the role of MET-PET in target volume definition of meningioma patients. In skull-base tumours they found MET-PET to be superior in defining tumour infiltration in the surrounding structures compared to CT/MRI. Furthermore, they demonstrated that MET-PET significantly decreased the interobserver variability in tumour delineation as compared to CT/MRI-based delineation<sup>[13]</sup>.

#### *Reduction of interobserver variability*

Interobserver variability of radiation target volume definition is a widely recognized problem. In laryngeal cancer for example, target definition using only CT leads to significant inter- and intraobserver variations in delineation of the GTV<sup>[14]</sup>.

When imaging modalities become more accurate, the interobserver variability will decrease. A reduction in the

interobserver variability is seen by incorporating FDG-PET data in the treatment planning<sup>[13,15–18]</sup>. Ciernik *et al.*<sup>[17]</sup> reported a reduction in the mean volume difference of 26.6 cm<sup>3</sup> to 9.1 cm<sup>3</sup> when FDG-PET was incorporated into the CT-based GTV definition of 39 patients with various solid tumours. This was associated with a reduction of the standard deviation from 38.6 cm<sup>3</sup> to 14.4 cm<sup>3</sup>. For 30 patients with NSCLC Caldwell *et al.*<sup>[16]</sup> found a large variation in GTVs between the observers. The mean ratio of largest to smallest GTV was 2.31 when CT based and 1.56 when PET-CT based, indicating a clear improvement. When analysing target definition of 22 patients with NSCLC by 11 observers, Steenbakkers *et al.*<sup>[18]</sup> found that the amount of disagreement was reduced from 45% (CT based) to 18% (PET-CT based). Ashamalla *et al.*<sup>[15]</sup> reported that for 19 patients with NSCLC the interobserver GTV variability decreased from a mean volume difference of 28.3 cm<sup>3</sup> (CT based) to 9.1 cm<sup>3</sup> (PET-CT based), with a respective



**Figure 2** Planning CT (A), corresponding FDG-PET (B) and fusion image (C) show differences in target volume definition. PET activity in the air cavity is also illustrated.

decrease in standard deviation from 20.99 to 6.47. Co-registration of CT and FDG-PET reduces this variability even further, which was demonstrated in NSCLC by comparing co-registered to non-registered images<sup>[19]</sup>.

### Thresholding

Studies comparing GTV definition using FDG-PET (GTVpet) and T1 weighted contrast enhanced MRI (GTVmri) in high-grade astrocytomas consistently showed that GTVpet was smaller than GTVmri<sup>[20,21]</sup>. Similar studies comparing GTVpet to delineation using CT or MRI (GTVct/mri) have been performed in head and neck cancer<sup>[17,22–26]</sup>. Results show either no difference between GTVs or that GTVpet was significantly smaller than GTVct/mri. A reason for this might be that the optimal way to delineate a tumour on PET is not clearly defined, resulting in variations in the methods for defining GTVpet. In clinical nuclear medicine, PET studies are usually interpreted qualitatively, whilst in radiation oncology a more quantitative approach is

necessary as edge detection is required for tumour contouring<sup>[27]</sup>.

PET images can be interpreted by visual assessment only (GTVvis), or by choosing thresholds, i.e. segmenting a lesion on the basis of a given level of radioactivity. This threshold could be any fixed cut-off of the standardized uptake value (SUV), and some investigators choose a cut-off of 2.5 (GTV2.5)<sup>[28,29]</sup>. Thresholds are more commonly defined as a fixed percentage of the maximum tumour activity, for example 40% (GTV40%). Erdi *et al.*<sup>[30]</sup> performed two-dimensional analyses of phantom experiments and stated that a threshold between 36% and 44% of the maximum activity would lead to an adequate segmentation. Ciernik *et al.*<sup>[17]</sup> recommended a fixed threshold of 50% of the maximum activity, also based on phantom data. A more sophisticated method developed at the University St Luc (UCL) in Brussels uses an adaptive threshold based on the signal-to-background ratio (GTVucl)<sup>[31,32]</sup>. This method aims to incorporate specific PET imaging properties by deriving a mathematical function from phantom-measurements

of objects of various sizes under various signal-to-background conditions.

Nestle *et al.*<sup>[28]</sup> recently compared these four PET delineation methods in 25 NSCLC patients. They observed substantial differences in the resulting GTVs and demonstrated that the choice of a tool for target volume definition based on PET images is far from trivial.

Examples of two cases where different delineation tools were applied are given in Figs. 1 and 2.

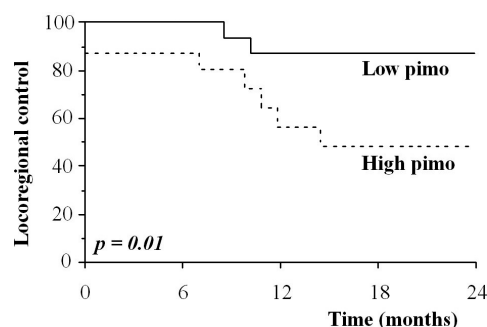
### Site specific issues

If a lung tumour causes atelectasis, it becomes extremely difficult to define the actual tumour on CT. In this situation most radiation oncologists would, when in doubt, prefer to define a larger target volume and possibly encompass part of the atelectasis rather than risk missing part of the tumour with a smaller target volume. FDG-PET may assist in more accurate definition of the border between tumour and atelectasis. This potential application of FDG-PET needs to be validated by resection specimen analysis. If this is proven to be reliable, radiation oncologists will confidently delineate a smaller target than they have done in the past. This can potentially result in a reduction of pulmonary toxicity by reducing the mean lung dose in patients currently eligible for high dose radiotherapy<sup>[33]</sup>. It also means that patients who previously were not eligible for this treatment because of pre-existent impaired pulmonary function, could then become eligible as a result of the reduction in expected radiation damage to the lung.

Several issues need to be addressed when using FDG-PET in radiation treatment planning of NSCLC. Although most primary NSCLC are visualized with FDG-PET, bronchoalveolar carcinoma shows limited or no increased FDG-uptake<sup>[34,35]</sup>. Furthermore, a post-obstruction pneumonia may also cause increased FDG accumulation, while malignant mediastinal involvement can be missed in those patients with relatively high FDG uptake in the heart. Issues associated with organ motion have already been addressed for CT scanning by gating image acquisition to the respiratory cycle. Similarly, it is possible to gate the linear accelerator. Gating the PET acquisition is also possible but experience is still relatively limited<sup>[36,37]</sup>.

In head and neck cancer, FDG-PET may miss small lesions with insufficient tumour cells to delineate the tumour, e.g. in superficial tumour infiltration of the mucosal lining<sup>[8]</sup> or micrometastatic disease in the regional lymph nodes. False positive findings can occur in inflammatory lymph nodes, tonsils, salivary glands and in areas of muscle activity (soft palate, base of tongue)<sup>[38]</sup>. When using segmented PET to delineate head and neck cancer, the GTV may become artificially enlarged by an inherent enclosure of part of an air cavity (Fig. 2). This needs to be corrected by using the CT as the anatomical

template and subsequently 'trimming' part of the GTVpet which is clearly in an air cavity. Thus, integration of FDG-PET into target volume definition is not trivial, but feasible. The GTVpet generally is smaller than the GTVct or GTVmri for the primary tumour. However, accuracy for primary tumour delineation has only been fully validated for nine laryngeal cancers. Further studies need to elucidate the best methodology for FDG-PET based tumour delineation.



**Figure 3** Influence of tumour oxygenation on outcome after radiotherapy for head and neck cancer. Tumour hypoxia was measured by administering pimonidazole followed by immunohistochemical staining. High pimonidazole binding (low oxygen tension) was associated with significantly worse outcome. Reprinted with permission from Kaanders *et al.*<sup>[40]</sup>.

## Can biological tumour characteristics be visualized?

### Relevant factors for treatment outcome

Tumour hypoxia is a strong contributor to radiation resistance<sup>[39]</sup>. Kaanders *et al.*<sup>[40]</sup> correlated the hypoxic fraction measured by staining tumour biopsies with pimonidazole, an exogenous hypoxia marker, with locoregional tumour control after radiotherapy of advanced head and neck cancer<sup>[40]</sup>. Tumour control inversely correlated with the hypoxic fraction (Fig. 3). Overgaard *et al.*<sup>[1]</sup> demonstrated in a large randomised placebo controlled trial that administration of the hypoxic cell radiosensitiser nimorazole to radiotherapy could improve loco-regional control and disease free survival in patients with head and neck cancer.

Various treatment modifications are available to counteract hypoxia induced radioresistance: irradiating during hyperoxic gas breathing under normobaric or hyperbaric conditions, adding a hypoxic cell sensitiser (nimorazole) or a hypoxic cytotoxin (tirapazamine), or increasing the radiation dose. To various extents these modifications lead to increased toxicity. As not every patient benefits from these treatment intensifications, careful selection of patients is necessary, and PET could be of value as a predictive tool.

Another tumour characteristic associated with radioreistance is tumour cell proliferation, especially in squamous cell carcinomas. This can be counteracted by shortening the overall treatment time, which has been shown to be effective in several randomised clinical trials<sup>[41,42]</sup>. As this is also a treatment intensification, PET could possibly help in selecting patients by identifying highly proliferating tumours, and thereby spare those patients who are not likely to benefit from the increased toxicity<sup>[43]</sup>.

Epidermal growth factor receptor (EGFR) inhibition is another strategy to counteract tumour cell proliferation. EGFR plays a key role in cellular proliferation of head and neck cancer, and Bentzen *et al.*<sup>[44]</sup> recently discovered that the amount of EGFR expression in tumour biopsies could reliably be used to select the dose fractionation scheme that had the greatest chance of benefiting the patient. Bonner *et al.*<sup>[45]</sup> reported that adding an EGFR-inhibitor (cetuximab) to the radiation treatment in a randomised clinical trial resulted in increased tumour control with only limited increase of toxicity.

### *Which radiopharmaceuticals are available to image these aspects?*

For imaging of tumour hypoxia both imidazole- and nonimidazole-containing agents have been developed. Imidazole containing radiopharmaceuticals are [<sup>18</sup>F]fluoromisonidazole (FMISO) and [<sup>123</sup>I]iodoazomycin arabinoside (IAZA). Non-imidazole tracers are <sup>99m</sup>Tc 4,9-diaza-3,3,10,10-tetramethyldodecan-2,11-dione-dioxime (<sup>99m</sup>Tc HL91) and <sup>64</sup>Cu-diacetyl-bis(*N*-4-methylthiosemicarbazone) (<sup>64</sup>Cu-ATSM).

Cell proliferation can be identified by labelling DNA precursors like thymidine or deoxyuridine, which are incorporated in DNA replication during the S phase of cycling cells. Clinical studies show a correlation between 3-deoxy-3-[<sup>18</sup>F]fluorothymidine (FLT) uptake and the Ki-67 labelling index. The latter is an accepted immunohistochemical marker to measure proliferation<sup>[46,47]</sup>. Further clinical validation of FLT against histopathological standards is in progress. At present, new radiopharmaceutical are developed in the preclinical phase to quantify tumour EGFR expression with PET, e.g. Ga-68-EGF and Zr-89- cetuximab<sup>[48,49]</sup>.

### *Validation*

FMISO was shown to bind selectively to hypoxic cells at radiobiologically relevant oxygen levels<sup>[50]</sup>. Piert *et al.*<sup>[51,52]</sup> demonstrated in pigs that FMISO retention occurred at relevant pO<sub>2</sub> levels after restricting blood supply to part of the liver. pO<sub>2</sub> levels were measured using invasive oxygen-sensitive Eppendorf histograph needle electrodes. In patients, hypoxia can also be

detected by the exogenous marker pimonidazole. Bioreduction and irreversible binding of this marker occurs at pO<sub>2</sub> levels below 10 mmHg and can be visualised by immunohistochemistry in tumour sections<sup>[53]</sup>. To validate the usefulness of FMISO for detection of hypoxia in human tumours, Troost *et al.*<sup>[54]</sup> compared FMISO autoradiography with pimonidazole immunohistochemistry in xenografted human squamous cell carcinomas and glioblastomas under various conditions of oxygen supply. They found a significant correlation between the pimonidazole derived hypoxic fractions and the mean FMISO signal intensity, but also noticed that the correlation varied between the tumour lines stressing the need for validation in clinical studies with different tumour entities (Fig. 4). Furthermore they observed that in the registration of changes of oxygenation status, FMISO was less accurate as the reference pimonidazole.

### *Temporal changes-treatment induced changes*

Little is known about the temporal stability of hypoxia imaging, and there are at least three aspects to consider. First, hypoxia can be transient due to structural and functional abnormalities of the tumour microvessels<sup>[55]</sup>. These abnormalities cause disturbances in the blood supply leading to temporal shutdown of vessels<sup>[55–57]</sup>. So, areas identified as ‘normoxic’ could be ‘hypoxic’ at a different time point. These changes occur at the microregional level and little is known about the sensitivity of PET-scanning for such changes. Second, the lifetime of chronically hypoxic cells is short, varying from a few hours to several days<sup>[58,59]</sup>. By the time radiation treatment commences, the hypoxic cells that were imaged will have already died and be replaced. Although the lifetime of individual cells is short, it is unlikely that the overall hypoxic pattern of a tumour will change significantly in a period of a few days. However, if intervals between PET imaging and treatment exceed one or two weeks, this may become a relevant problem. Third, irradiation itself can cause rapid changes in oxygenation and perfusion<sup>[60]</sup>.

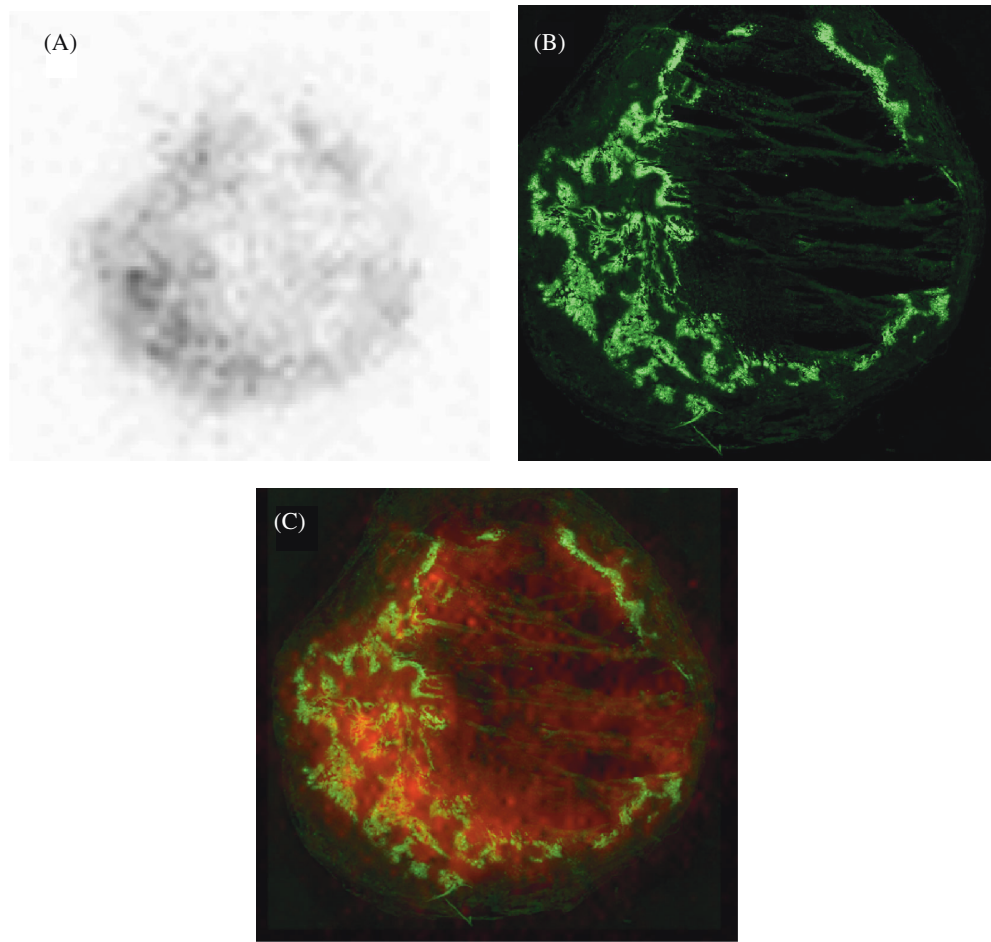
So even if the information obtained by functional imaging correlates with a relevant tumour characteristic, and even if that characteristic has an impact on clinical decision making on treatment selection, one has to be aware that the temporal stability of the imaged data may be limited. Currently, studies of temporal stability of hypoxia maps are in progress.

## **Can intratumoural biological heterogeneity be identified?**

### *Concept of dose painting*

The concept of dose painting was proposed by Ling *et al.*<sup>[2]</sup>. The idea is to visualize tumour subvolumes with





**Figure 4** FMISO autoradiography (A), pimonidazole immunohistochemistry (B) and fusion image (C) of a xenografted human head and neck cancer. Courtesy of E. Troost, Department of Radiation Oncology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

a potential resistance to irradiation and to paint some additional dose onto that volume. Chao *et al.*<sup>[61]</sup> applied this in a pilot study demonstrating an IMRT plan where a subvolume of the oropharyngeal tumour, identified by increased  $^{64}\text{Cu}$ -ATSM retention, received an extra dose of 10 Gy. Despite the dose escalation in this dose painting exercise the parotid glands could still be adequately spared with the high precision IMRT technique.

### *Spatial resolution*

At the level of the tumour microenvironment, it is conceivable that new cells formed in the proliferating cell compartment push older cells away from the blood vessels resulting in a gradual depletion of oxygen and nutrients. Tumour cells in the hypoxic compartment would be pushed further down the oxygen gradient and eventually die of oxygen deficiency and starvation<sup>[62]</sup>. The dynamics of hypoxic tumour cells in xenografted human head and neck cancer was analysed by consecutive injection of two different hypoxia markers<sup>[59]</sup>. Over time, pimonidazole positive cells were being pushed away

from the vasculature and cell debris with pimonidazole adducts appeared in the necrotic regions. Meanwhile, new hypoxic cells appeared at the 'hypoxic front' identified by a second marker, CCI-103F, administered at a later point in time. This 'pattern of hypoxia' is measured in micrometres and therefore cannot be detected by *in vivo* imaging techniques such as PET due to limited spatial resolution. So PET images hypoxia at a more global level. Furthermore the radiation dose delivery also has a certain resolution. Given these limitations, dose painting will most likely only be feasible with subvolumes greater than  $0.5\text{ cm}^3$ . PET imaging would then have to identify subvolumes within the tumour with a larger than average content of hypoxic cells.

### **Conclusion**

Integrating biological or molecular information of tumours into radiation oncology might help in deciding not only *where* but also *how* radiation therapy should be delivered<sup>[63]</sup>. The addition of functional imaging to the standard anatomically based target volume definition has



already shown significant advantages, especially when images are co-registered. The reduction of interobserver variability is obvious and can increase the standard of care for all patients, not only by reducing geographically missing parts of the cancer, but also by reducing the irradiated volume of normal tissues and organs at risk.

As more studies become available validating the various functional imaging properties, more treatment related decisions (dose painting, shortening overall treatment time, adding a sensitizer) will have to be tested in clinical trials. The goals are challenging and clear: first, to enlarge the therapeutic window by increasing the tumour control probability and decreasing the normal tissue complication probability; second, to develop predictive assays that can serve as selection tools for patients that are likely to profit from intensified treatments. We believe that molecular imaging can play an important role in achieving these goals.

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